Application of the Exposure Related Dose Estimating Model (ERDEM) in Support of the Food Quality Protection Act (FQPA) for the Assessment of Aggregate Exposure of Infants and Children to Organophosphorus Insecticides Used in Residential Environments

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1 INTRODUCTION

The implementation of FQPA requires EPA to consider the combined or aggregate effects of pesticide exposure from food, drinking water, and other non-occupational uses (1). To evaluate the risks from inhalation, dietary, and dermal exposure a PBPK model was developed within the ERDEM framework (2,3). Conservative assumptions underlying residential exposure and cumulative risk were examined as inputs for particular exposure scenarios using residential dermal transfer coefficients, ambient air concentrations and dietary intake (4,5). Physiological, pharmacokinetic and pharmacodynamic parameters describing the fate of parathion, isofenphos and chlorpyrifos in animal systems were scaled to establish the model structure for human exposure. Adjustments were made for differences in metabolism and physiology between children and adults. One age-group was evaluated (children 1-3 yrs of age, 10 kg) reflecting children in contact with the treated surfaces, ingesting residues on hands and in food, and inhaling residues in the air. Bimolecular rate constants, ki ($pM^{-1}\ hr^{-1}$) were used to describe inhibition of the "B"-esterases (acetylcholin-, butyrylcholin- and carboxyl-) by toxic oxons. Michaelis-Menten kinetics were used to describe the activation of parathion, chlorpyrifos and isofenphos to their corresponding oxons followed by hydrolysis of parent chemicals and oxons to yield biomarkers of metabolism. Rates for enzyme synthesis, reactivation and aging were used in blood, liver and brain to account for depletion and replacement of the enzymes.

2 METHODS

PBPK/PD Model, ERDEM. Figure 1 gives the flow chart for the ERDEM framework (2).

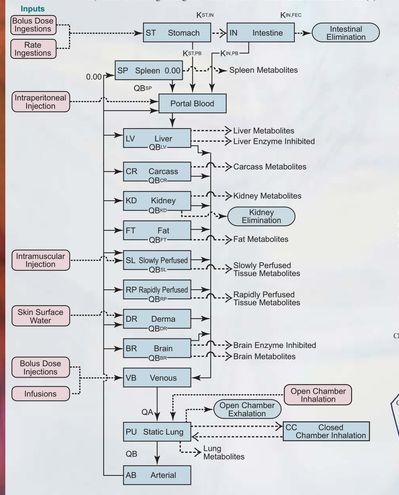


Figure 3. Combined metabolic pathway for Isofenphos in the rat, guinea pig and dog (3). (1) IF, isofenphos to IO, isofenphos oxon; (2) IF to DNI; des N-isopropyl isofenphos; (3) IO to DNIO, des N-isopropyl isofenphos oxon; (4) DNI to DNIO, (5) DNI to DAI, desaminoisofenphos, (6) DNIO to DAIO, des aminoisofenphos oxon; (7) IO to AP1, O-ethylisopropylaminophosphate and IPS, isopropyl salicylate; (8) DNIO to AP2, O-ethylaminophosphate and IPS; (9) DAIO to AP3, O-ethyl-phosphate and IPS; (10) DNI to AP4, O-ethylamino-thiophosphate and IPS; (11) DAI to AP5, O-ethylthiophosphate and IPS; (12) IF to IFA, carboxylic acid of isofenphos; (13) DNI to DNIA, carboxylic acid of DNI; (14) DAI to DAIA, carboxylic acid of DAI; (15) IO to IOA, carboxylic acid of IO; (16) DNIO to DNIOA, carboxylic acid of DNIO; (17) DAIO to DAIOA, carboxylic acid of DAIO; (18) IPS to SA, 2-hydroxy salicylic acid; (19) SA to 2-OH-HA, 2-hydroxy hippuric acid.

The metabolic pathways (Figures 2, 3, and 4 for parathion, isofenphos and chlorpyrifos) are presented in Figures 2, 3, and 4 respectively.

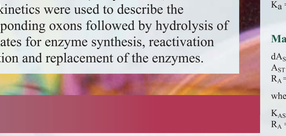
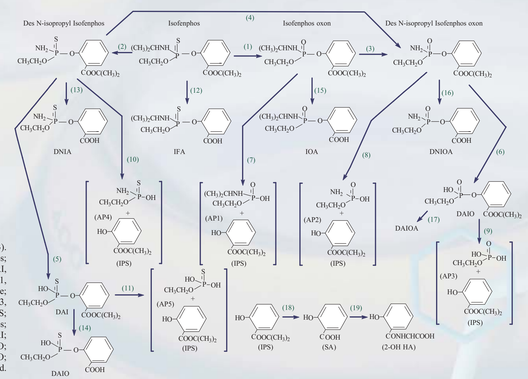


Figure 2. Metabolic pathway for parathion in the rat. (1) parathion to paraoxon, (2) parathion to O,O-diethyl phosphorothioate and p-nitrophenol, (3) paraoxon to O,O-diethyl phosphate and p-nitrophenol, (4) p-nitrophenol to p-nitrophenyl sulfate and (5) p-nitrophenol to p-nitrophenyl glucuronide.



Mass Balance for Percutaneous Absorption. The percutaneous absorption mass balance equation for simulating dermal absorption of pesticide residues is given below:

$dA_{surf}/dt = K_p * A * (C_{sk}/P_{a/sk} - C_{surf}) - K_A A_{surf}$, mmol h^{-1}
$A_{surf} = Integ\ (dA_{surf}/dt, 0.0)$
$C_{surf} = A_{surf}/V_{sk}$, mmol cm^{-3}
$C_{sk} = A_{sk}/V_{sk}$, mmol cm^{-3}
$dA_{air}/dt = K_A * A_{surf}$, mmol h^{-1}

where:

K_P = skin permeability constant, cm h^{-1}
A = Area of treated or exposed skin, cm^2
$P_{a/sk}$ = air/skin partition coefficient
K_A = evaporation rate

Mass Balance for Gastrointestinal Absorption.

$dA_{ST}/dt = -K_{AS} * A_{ST}$, Change in the amount in stomach, mmol h^{-1}
$A_{ST} = Integ\ (R_{ST}, ODOSE)$, Amount in stomach, mmol
$R_A = K_{AS} * A_{ST}$, Rate of absorption, mmol hr^{-1}

where:

K_{AS} = Absorption constant in h^{-1}
R_A = Rate of absorption

Mass Balance for Inhalation.

Amount of Pesticide in Blood
$C_{A1} = (QP * C_I + QC * CV) / (QC + (QP/PB_1))$

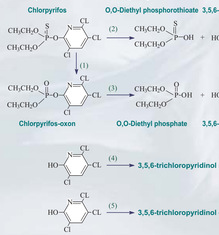
Amount of Pesticide Inhaled
$R_{AI} = QC * (C_{AI} - C_A)$
$A_{LU} = Integ\ (R_{AI}, 0.0)$
$C_{LU} = A_{LU}/V_{LU}$
$C_A = C_{LU}/P_{LU}$

Amount of Pesticide Exhaled
$R_{AX} = QP * C_X$
$A_X = Integ(R_{AX}, 0.0)$
$C_X = C_{A1}/P_{B1}$

where:

C_I = Concentration Pesticide in Inhaled air, mmol L^{-1}
C_X = Concentration of Pesticide in Exhale air, mmol L^{-1}
R_{AI} = Rate Pesticide is inhaled, mmol hr^{-1}
A_I = Amount of Pesticide Inhaled, mmol
C_A= Concentration in arteriole blood leaving the lung, mmol L^{-1}
R_{AX} = Rate Pesticide is exhaled, mmol hr^{-1}

Figure 4. Metabolic Pathway for parathion in the rat. (1) chlorpyrifos to chlorpyrifos-oxon, (2) chlorpyrifos to O,O-diethyl phosphorothioate and TCPy, (3) chlorpyrifos-oxon to O,O-diethyl phosphate and TCPy, (4) TCPy to sulfate, and (5) TCPy to glucuronide.



Mass Balance for OP pesticides in Blood. Mass balance equations were written for venous and arteriole blood involving the absorption of OP pesticides from the stomach, skin and lungs, transfer to tissues, metabolism of parent OP in the liver, metabolism of oxons in liver and blood and inhibition of one or more "B" esterases (AChE and BChE) in blood by oxons.

Mass Balance for OP pesticides in Liver. Mass balance equations were written for the liver with OP pesticides absorbed via the stomach, skin and lungs metabolizing to the ith metabolite, loss of metabolites (oxons) due to inhibition of one or more "B" esterases, and losses due to binding and elimination.

Mass Balance for OP pesticides in Brain. Mass balance equations were written for the brain involving the metabolism of circulating oxons, and phosphorylation of AChE, CaE and BChE by oxons.

Mass Balance for "B" esterases in Blood, Liver and Brain. Mass balance equations were written for the inhibition of "B" esterases in blood (AChE and BChE), liver (BChE and CaE) and brain (AChE, BChE and CaE). Equations involving degradation and aging of phosphorylated enzymes and resynthesis of new enzymes were included.

Inhibition of Tissue AChE, ChE, and CaE by Toxic Oxons. In the isofenphos, parathion and chlorpyrifos PBPK/PD models, "B"-esterases [blood AChE and BChE, brain AChE, BChE and CaE, and liver CaE and BChE] were inhibited by des N-isopropyl isofenphos oxon (DNIO), paraoxon and chlorpyrifos-oxon. The bimolecular reaction constants (k_i) were calculated in the model using literature values for K_i and k_r (or k_{-2}), where $k_i = K_i/k_r$ (k_{-2}). The mass balance equation for the inhibition of blood AChE by either DNIO, paraoxon or chlorpyrifos-oxon and recovery of AChE activity is given below:

$V_B * dA_{ACHEB}/dt = (K_{iACHEB} * C_{ACHEB} * C_{DNIOB}) - (K_{RACHEB} * A_{iACHEB})$, mmol h^{-1}

where:

V_B = Volume of blood, L
A_{iACHEB} = Inhibited AChE, mmol
K_{iACHEB} = AChE bimolecular inhibition rate constant, (mmol $L^{-1})^{-1}\ h^{-1}$
C_{ACHEB} = Concentration of Free AChE in blood, mmol L^{-1}
C_{DNIOB} = Concentration of DNIO/paraoxon/chlorpyrifos in the blood, mmol L^{-1}

K_{RACHEB} = Rate of reactivation of inhibited AChE (h^{-1})

Exposure Inputs to ERDEM. Table 1 lists the exposure inputs for children's dermal, oral, and inhalation into ERDEM for isofenphos, parathion, and chlorpyrifos (4,5).

Table 1. Exposure scenarios involved 1-3 year old children (10 kg) having a total body surface of 4325 cm^2. Dermal exposures occurred twice a day for duration of 1.5-2.0 hr, oral exposures (residues in food) took place at breakfast, lunch and dinner for a period of 0.5 hr, while oral exposure to isofenphos skin residues occurred during dermal exposure. Inhalation of chlorpyrifos (1.5-2.0 hr) occurred during dermal exposures. Exposures were repeated (simulated) on a daily basis for 3 and 12 consecutive days.

Pesticide	Exposure Type	Specified Exposure	Concentration as Input to ERDEM	Area or Volume	Start/Duration
Isofenphos	Dermal	0.5 µg/cm² of Surface Area	1.488E-3 mm/L	4325 cm^2	10:00/2 hr 15:00/2 hr
Isofenphos	Oral	0.5 µg/cm² of Surface Area	6.8E-4 mm/L	94 cm^2	10:00/2 hr 15:00/2 hr
Parathion	Oral	1.67 µg/kg of volume	1.147E-3 mm/L	10 kg	07:00/0.5 hr 12:00/0.5 hr 17:00/0.5 hr
Chlorpyrifos	Dermal	0.18 µg/cm² of Surface Area	5.134E-4 mm/L	4325 cm^2	07:30/1.5 hr 13:00/2.0 hr
Chlorpyrifos	Oral	0.333 µg/kg	1.8997E-4 ppm	10 kg	07:00/0.5 hr 12:00/0.5 hr 17:00/0.5 hr
Chlorpyrifos	Inhalation	0.15 ng/L	1.0468E-4 ppm	N/A	07:30/1.5 hr 13:00/2.0 hr

3 RESULTS

Metabolic Fate of Isofenphos, Parathion and Chlorpyrifos in a 10 kg child

Table 2 gives the fate of absorbed doses (mmoles and µg/kg) of isofenphos, parathion and chlorpyrifos.

Pesticide	Route	Dose in mmoles and µg/kg	% of Dose in Tissues	% of Dose Metabolized	Urinary Metabolites % of Dose
Isofenphos	Oral	8.16E-4	12.77; 7.5% in fat	87.2	IFA, 5.8% DNIA, 53.2% OH-HA, 21.9% SA, 3.2% IPS, 3.1%
	Dermal	1.19E-3 69.3 µg/kg total dose			
Parathion	Oral	5.16E-4 15.0 µg/kg total dose	11.5; 0.69% in fat	88.5	PNP, 1.0% PNPS, 80% PNPG, 7.5%
Chlorpyrifos	Oral	8.55E-4	4.50; 4.07% in fat	32.2; with 63% exhaled	TCP, 1.0% TCPG, 31.2%
	Inhalation	1.37E-3			
	Dermal	1.17E-3 92.1 µg/kg total dose			

IFA = isofenphos acid
DNIA = des N-isofenphos acid
OH-HA = 2-hydroxyhippuric acid
SA = salicylic acid
IPS = isopropyl salicylate

PNP = p-nitrophenol
PNPS = p-nitrophenyl sulfate
PNPG = p-nitrophenyl glucuronide
TCP = 3,5,6-trichloro-2-pyridinol
TCPG = 3, 5,6-trichloro-2-pyridinol glucuronide

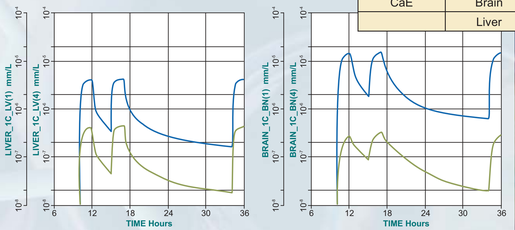
Cholinesterase Inhibition. Table 3 gives the predicted inhibition of AChE, BChE and CaE in blood, brain and liver of children after 3 days (72 hr) of repeated exposure to the aggregate residues (Table 1) of parathion, isofenphos and chlorpyrifos.

Pesticide		Amount of Inhibited AChE	Amount of Inhibited BChE	Amount of Inhibited CaE
Initial Amount of Enzyme	Venous Blood	4.4E-7 mmoles	1.92E-6 mmoles	
	Brain	5.7E-6 mmoles	1.91E-6 mmoles	8.25E-5 mmoles
	Liver		2.88E-6 mmoles	1.59E-4 mmoles
Isofenphos	Venous Blood	0.014%	0.19%	
	Brain	0.28%	0.37%	2.78%
	Liver		0.18%	1.35%
Parathion	Venous Blood	0.79%	1.0%	
	Brain	2.1%	2.9%	0.71%
	Liver		8.81%	2.30%
Chlorpyrifos	Venous Blood	0.29%	0.39%	
	Brain	0.31%	0.41%	0.10%
	Liver		3.4%	0.87%

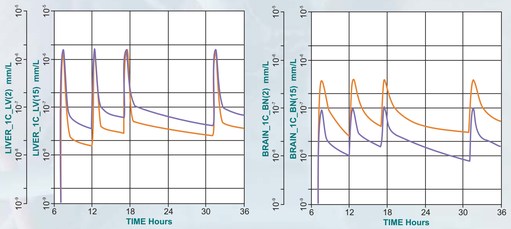
Table 4 gives the results after 12 days of exposure. Significant enzyme inhibition occurred in the liver.

Enzyme	Compartment	Starting Amount in pmoles	Ending Amount in pmoles	Percent Change
AChE	Blood	440.0	430.0	-2.7%
	Brain	5670.0	5530.0	-2.47%
BChE	Blood	1920.0	1869.06	-2.7%
	Brain	1905.0	1840.0	-3.4%
	Liver	2880.0	2470.0	-14.2%
CaE	Brain	82500.0	80900.0	-1.9%
	Liver	159200.0	150800.0	-5.3%

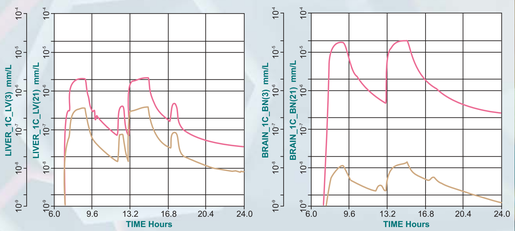
Concentrations of Parent and Oxons in Liver and Brain Resulting from Exposure



Isofenphos, oral and dermal exposure. Figures 5 and 6 give the concentrations of **isofenphos** and **des N-isofenphos** oxon in 10^{-5} to 10^{-7} mmols/L of liver and brain in a 10 kg child resulting from dermal exposure to 0.5 µg/cm^2 (4325 cm^2) for 2 h starting at 10:00 and going to 15:00 hr; and oral exposure to these residues on 94 cm^2 of skin. The des N-isopropyl isofenphos oxon concentration (peak) in brain (2.0E-7 mmoles) was less than the peak concentration in liver. The wide peak width resulted from receiving an oral and dermal dose of isofenphos.



Parathion, oral exposure. Figures 7 and 8 give the concentrations of **parathion** and **paraoxon** in 10^{-5} to 10^{-7} mmols/L of liver and brain in a 10 kg child resulting from the administration of 1.67 µg/kg of bw, three times a day (7:00, 12:00 and 17:00 hr). Paraoxon in the brain was 1/10 of the peak concentration predicted in liver.



Chlorpyrifos, oral, dermal and inhalation exposure. Figures 9 and 10 give the concentrations of **chlorpyrifos** and **chlorpyrifos-oxon** in 10^{-8} to 10^{-5} mmols/L of liver and brain in a 10 kg child resulting from dermal (0.18 µg/cm^2, 4325 cm^2, 1.5-2.0 hr), oral (0.333 µg/kg, 0.5 hr) and inhalation (0.15 ng/L of air, 1.5-2.0 hr) exposure at the times indicated in Table 1. Chlorpyrifos-oxon in brain was 1/300 of the peak concentration in liver. Here again, the wide peaks result from receiving dermal, oral and inhaled doses of chlorpyrifos.

4 CONCLUSIONS

1. An ERDEM PBPK/PD model was found capable of handling multiple inputs (aggregate OP exposures) and assessing their effects on target enzymes (cumulative effects).
2. The individual exposure levels (0.069, 0.015 and 0.092 mg/kg-day for **isofenphos**, **parathion** and **chlorpyrifos**) were above or slightly below the chronic NOEL exposure levels of 0.03 mg/kg-day for chlorpyrifos, and 0.05 mg/kg-day for **isofenphos** and **parathion**. The aggregate exposure level (0.2 mg/kg-day) was above the individual cholinesterase NOEL levels of **isofenphos**, **parathion** and **chlorpyrifos**.
3. No significant inhibition was observed in blood and brain tissue after 3 and 12 days of exposure. Significant inhibition was observed with liver enzymes.
4. The exposure levels selected in this exercise were above those estimated by Rigas et al. from the Minnesota Study (6).

5 REFERENCES

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